CHAPTER 16

Internal Standards for Organic Chromatographic Methods

16-1. Introduction.

For organic analyses, internal standards are compounds that are similar in chemical composition to the analytes of interest. However, unlike surrogates, internal standards are spiked into all instrument QC, batch QC, and environmental samples immediately prior to instrumental analysis. (Surrogates are spiked into batch QC and environmental samples prior to sample preparation and analysis.) For environmental applications, the internal calibration technique is typically used for mass spectrometry methods but may also be used for chromatographic methods with 2-D detectors. Internal standard response should be monitored throughout instrumental analysis to help evaluate instrument performance (e.g., sensitivity and stability) and matrix effects.

16-2. Criteria.

16-2.1. Frequency.

When quantitation is performed using internal standards, known quantities of internal standards must be added to all instrument QC, batch QC, and environmental samples immediately prior to instrumental analysis.

16-2.2. Acceptance Criteria.

a. The compounds used for internal standards, the concentrations, and the acceptance criteria will be highly dependent upon the analytical technique and the set of analytes of interest. In general, instrumental response for an internal standard must fall well within the calibration range. Internal standards in all samples must fall within the retention time windows for the most recent CCV (especially, for 2-D chromatographic methods). Ideally, internal standards should also elute at retention times that are near the retention times of the associated target analytes.

b. Unless a more appropriate criterion is available, the peak area for each internal standard in all instrument QC, batch QC, and environmental samples should be within -50% to +100% of the corresponding peak area for the mid-level initial calibration standard. The mean internal standard peak area for the set of initial calibration standards may be used in lieu of the internal standard peak area of the mid-level initial calibration standard.

> Note: This is contrary to the guidance presented in the "USEPA Contract Laboratory Program National Functional Guidelines for Organic Data Review." Internal standard peak areas for samples are evaluated using the internal standard peak area for the most recent CCV rather than the internal standard peak areas for the initial calibration. For example, for the "Volatile Data Review," Section X ("Internal Standards") states: "Internal standard area counts must not vary more than a factor of two (-50% to +100%) from the associated 12hr calibration standard." This practice is not recommended since it is based on the assumption that sensitivity, as measured by the internal standard areas

of the CCVs, will not progressively degrade (i.e., decrease) during the analysis. In other words, it is assumed that the internal standard peak area of each CCV will not systematically be significantly smaller than that for the preceding CCV.

16-3. Evaluation.

Review the internal standards summary form(s) and ensure that all the internal standards fall within the appropriate retention time windows and the internal standard areas fall within appropriate acceptance limits. Verify, using at least one of the CCVs, that the internal standard peak area acceptance limits were correctly calculated.

16-4. Qualification.

a. In general, environmental samples must be qualified for an unacceptable internal area count even when the samples are bracketed by acceptable CCVs.

Note: The composition of internal standards and the target analytes are similar but, in general, it should not be assumed that internal standards will behave in an identical manner as all the target analytes during all environmental conditions. For example, a matrix interference for a particular environmental sample may affect the internal standards more than the associated target analytes (e.g., the preferential absorption of an internal standard relative to the associated target analytes). Because of differences in volatility during purge-and-trap analyses, an internal standard may be lost more readily than its associated target analytes. For example, because of a poor seal for one of the purge vessels, the internal standard bromochloromethane can be lost more readily than the associate target analyte acetone.

b. The concentration of a target analyte is inversely proportional to the internal standard area. Hence, an unacceptably low area count tends to give rise to a high bias and an unacceptably high area count tends to give rise to a low bias. However, the analyte concentration is ultimately dependent upon the response for the internal standard as well as that for the target analyte. Therefore, the evaluation of bias on the basis of internal standard response is often problematic when a comprehensive data package is not available. If the internal standard area count of a sample does not fall within the acceptance range for internal standard area of the mid-level initial calibration, then qualify the results as summarized in the table below. It is assumed that a high internal standard response gives rise to a low bias. However, in order to obtain a more conservative evaluation of the data, a direction of bias is not assumed for low internal standard response.

c. An internal standard peak area that does not meet the threshold criteria for a detection may give rise to a false negative for the associated target analyte and surrogate results. If an extremely low area count is reported and the chromatograms are not available for review (e.g., to assess signal to noise ratios), a conservative approach must be used; qualify nondetections with the R flag. However, if a more detailed review of the data is planned, then the X flag would be more appropriate.

d. A more detailed review of the data is recommended when a sample's internal standard does not fall within the retention time acceptance windows. For example, the data evaluator should request a comprehensive data package and the raw data (e.g., chromatograms and quantitation reports) should be examined to determine if any false positives or false negatives exist.

Table 16-1
Data Qualification of Internal Standard Areas [1]

Internal Standard Area of Sample (A)	Sample Result (*y*)	Flag
½ $A_0 \leq A \leq 2\ A_0$	MRL < MQL < *y*	None
	MRL < *y* < MQL	J
	y < MRL	U
2 $A_0 < A < 5\ A_0$	*y* > MRL	J-
	y < MRL	UN
$(A_0/5) < A <$ ½ A_0	*y* > MRL	J
	y < MRL	U
A > 5 A_0	*y* > MRL	J- X if *y* < AL
	y < MRL	R
A < $(A_0/5)$	*y* > MRL	X
	y < MRL	R

Notes: 1.I t is assumed that the LOI ≤ MRL < AL. The following abbreviations are used: A = Internal standard area count sample; A_0 = Internal standard area count sample; y = Concentration of a target analyte for the field sample; AL = Action Level; MRL = Method Reporting Limit; MQL = Method Quantitation Limit.